Classification of Ovarian Stages of Walleye Pollock (*Theragra chalcogramma*)

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Abstract

Walleye pollock, *Theragra chalcogramma*, is the most commercially important species in the eastern Bering Sea. Estimates of maturity are critical for setting an appropriate harvest rate for pollock spawning biomass, and correct classification of ovaries into a maturity condition is necessary for accurate estimation of maturity. Data on length, weight, and ovary weight and condition were collected from 4,996 pollock in 2002 and 5,201 in 2003 aboard pollock trawlers across the eastern Bering Sea. In 2003, 173 pollock ovaries were collected and prepared for histological analysis. Maturity condition was assessed by macroscopic inspection of gonads, a gonado-somatic index (GSI), and histological methods. Macroscopic inspection was based on alterations in ovary size and appearance, whereas histological methods evaluated changes in oocyte stages. The GSI was calculated as the proportion of ovary to body weight. The GSI was a good indicator of pollock that had spawned or were about to spawn. Histological analysis confirmed the overall general appropriateness of macroscopic staging for mature versus immature fish, but it also indicated relatively high misclassification rates for particular maturity stages when using macroscopic staging methods alone. Among ovaries macroscopically classified as developing, 16% were at immature oocyte stages and 84% were at primary yolk to more advanced oocyte stages. This indicates that pollock classified macroscopically as "developing" may mature in either the current or subsequent spawning season. Macroscopic inspection and

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GSI in combination may be useful to determine maturity condition for some maturity stages; however, histological examination of ovaries is the most accurate method for all stages.

Introduction

Correct classification of ovaries into developmental stages is necessary to determine maturity status, a prerequisite for setting annual catch quotas using a harvest rate strategy based on spawning biomass estimates. Maturity condition during the spawning period may be identified by macroscopic inspection of gonads, a gonado-somatic index (GSI), histological methods, and/or oocyte (egg) size. Macroscopic inspection is based on alterations in ovary size and appearance, whereas histological methods evaluate changes in oocyte stages at the cellular level. The GSI is calculated as the proportion of ovary to body weight (Gunderson and Dygert 1988); female ovary and body weight increase in advance of spawning due to absorption of ambient water (Sakurai 1989). However, some loss in ovary weight occurs as each batch of eggs is spawned (Teshima et al. 1989).

Classification of pollock ovaries is confounded, because oocytes are found at several different developmental stages within an individual ovary. By convention, the most advanced stage is used for classification (Hinckley 1987). The proportions of oocytes at each stage do not vary with location in the ovary (Teshima et al. 1989), but the proportions of oocytes at particular stages change with the progression of the reproductive cycle (Hinckley 1987).

In the recovery or transitional state of the reproductive cycle, oocytes are found only in a yolkless condition (Sakurai 1989). However, throughout the reproductive cycle, a reserve of yolkless oocytes exists in early and late peri-nucleus stages, called the "reserve fund" (Hinckley 1987). A group of oocytes advance asynchronously from the "reserves" and begin yolk formation (vitellogenesis). Once the entire group has reached the tertiary yolk stage, vitellogenesis is complete, and no more "reserves" will be recruited for development in the approaching spawning season (Hinckley 1987). At this stage the fully yolked oocytes and "reserves" are separated by a bimodal size distribution (Sakurai 1989). Batches of fully yolked oocytes further develop synchronously into mature hydrated oocytes, first undergoing yolk coalescence and then hydration (Sakurai 1989). Oocytes become enlarged and transparent with the uptake of water during hydration causing the size and weight of the ovary to greatly expand. Oocytes are then discharged from their follicles and become ova during ovulation (Bowden et al. 1990). Finally, the batch of oocytes is spawned. Subsequently, additional groups of ova may undergo this process in the same spawning season, a repetitive process called batch spawning (Hinckley 1987). Atresia, or the

resorption of oocytes, may occur at low frequencies to remove residual oocytes that are abnormal or damaged or to adjust the number eggs spawned in a batch (Bromley et al. 2000). In Atlantic cod (*Gadus morhua*) high frequencies of atresia may result from poor nutrition associated with prey type and cessation of feeding (Rideout and Rose 2006) or water temperatures too cold for gamete development (Rideout et al. 2000). Mass atresia has also been attributed to low water temperature for Greenland halibut (*Reinhardtius hippoglossoides*; Federov 1971) and haddock (*Melanogrammus aeglefinus*; Hodder 1965).

Spent ovaries are identified by the combined presence of postovulatory follicles and oocytes in only early and late peri-nucleus stages (Hinckley 1987). Follicles are composed of an inner layer of cube-shaped granulosa cells and an outer layer of elongate thecal cells containing blood capillaries. The follicle surrounds the oocyte and serves to transport nutrients, wastes, and yolk proteins between the oocyte and the maternal bloodstream. After ovulation, the postovulatory follicle may appear as a convoluted structure (Hunter and Macewicz 1985).

Our objectives were to (1) confirm the appropriateness of macroscopic staging of ovaries with histological examination, (2) produce an accurate and understandable descriptive guide for future macroscopically staging of ovaries based on histology, and (3) investigate the usefulness of GSI for predicting maturity condition for walleye pollock in the eastern Bering Sea. Successful completion of these objectives allows subsequent evaluation of current estimation methods for pollock spawning biomass from which annual catch quotas are set for the eastern Bering Sea.

Methods

Maturity stage samples

Maturity data were collected by Pollock Conservation Cooperative (PCC) member vessels during the "A" roe season of 2002 and 2003 from late January to early April over the full geographic area fished by the participating PCC fishing vessels (Fig. 1). Vessels were highly concentrated north of Unimak Island and followed the 100 m depth contour beyond the Pribilof Islands. Ten female pollock from one haul per day per vessel were sampled from a range of lengths, and if possible, no more than 10 fish were taken from one 10 cm length category per week. This procedure assures an adequate size range of fish for estimation of maturity schedules. Each female fish was sampled for fork length, body weight, ovary weight, and maturity stage, based on macroscopic visual observation with criteria from NMFS five-stage scale (Table 1).

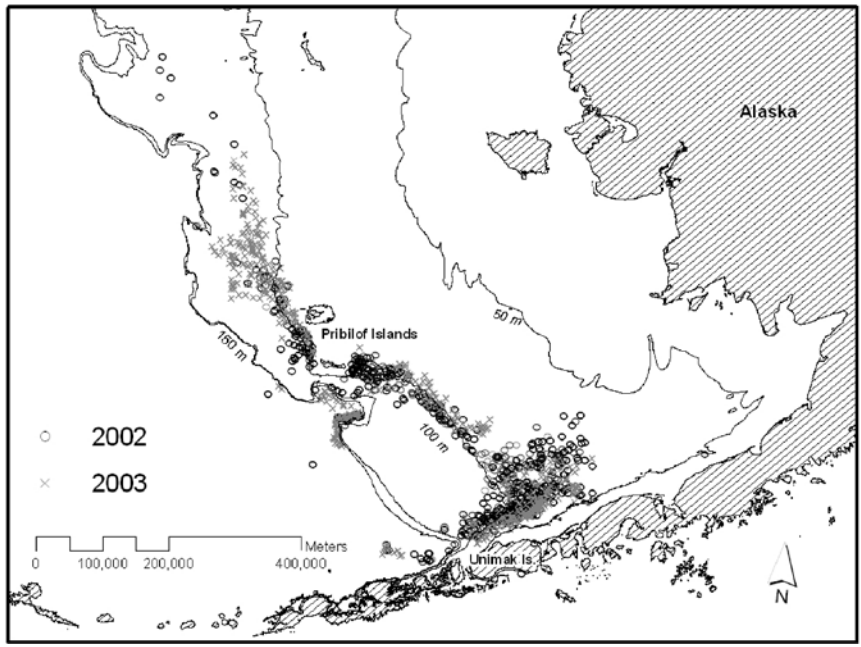

Figure 1. Distribution of Pollock Conservation Cooperative member pollock trawling vessels in 2002 and 2003 "A" fishing season (January 20-March 30 in 2002 and January 21-April 2 in 2003). Each symbol (X or O) represents a location where one or more net hauls occurred and pollock maturity data were collected.

Ovary tissue samples

We collected ovaries and maturity data from 173 female pollock aboard the 115 m midwater trawler F/V *Alaska Ocean* during normal fishing operations during February 22-28, 2003. Ovaries were sampled at all available developmental stages, with an emphasis on small, less-developed ovaries to reconcile uncertainty of macroscopic classification at early developmental stages. Because the proportion of hydrated oocytes increases toward the center of the ovary (Teshima et al. 1989), tissue was cut from one location at the midsection of each ovary (Bowden et al. 1990) to obtain the most advanced oocyte stage present. Tissues were placed in slotted microcassettes of approximate size 2.5 cm × 3.8 cm and stored in a solution of 1 part formalin and 9 parts water buffered with 20 g per L sodium acetate. In addition, fork length (nearest cm), ovary weight (nearest g), body weight (g) without stomach contents but

Table 1. Pollock maturity condition based on macroscopic examination. A five-stage scale was used by Pollock Conservation Cooperative member vessels to macroscopically stage female pollock ovaries; criteria were based on NMFS five-stage scale.

Maturity code	Condition	Macroscopic examination
1	Immature	Ovary transparent with no eggs visible. Gonad small and tucked inside body cavity.
2	Developing	Ovaries translucent to opaque, and about half the length of body cavity. Spawn within following year.
3	Pre-spawning	Ovaries orange, reddish, and occupy about 2/3 of body cavity. Eggs are discernible and opaque.
4	Spawning	Roe runs with slight pressure. Most eggs are hydrated (translucent) with few opaque eggs.
5	Spent	Ovaries empty and flaccid.

with ovaries and liver were measured, and for each fish maturity stage was estimated visually based on the five-point scale (Table 1). Ovary samples were embedded in paraffin, thin sectioned to a thickness of about 4 µm, and stained and counterstained with H&E at Phoenix Labs, Inc., Everett, Washington.

Laboratory and data analyses

Histological analysis was performed to confirm the accuracy of macroscopic determinations of maturity. The entire slide of each tissue sample was scanned with a compound microscope. Oocytes were photographed at each developmental stage (Figs. 2-10) and were classified into developmental stages according to criteria and published photographs (Hinckley 1987, Sakurai 1989). In addition, pollock ovary tissue was examined for atretic oocytes and the presence of postovulatory follicles (Fig. 10).

Pollock ovaries were classified to maturity stage based on the most advanced oocyte stage present (West 1990) and on the presence or absence of postovulatory follicles (Hinckley 1987). Criteria are shown in Table 2 with corresponding reference photographs (Figs. 2-10). Ovaries were categorized as pre-spawning if their most advanced oocytes were at primary yolk or more advanced stages with no empty follicles present. This classification is based on the presumption that walleye pollock oocytes develop from the primary yolk stage to spawning in four months (Y. Sakurai, Hokkaido University, pers. comm.). Ovaries were categorized as spawning if postovulatory follicles were present.

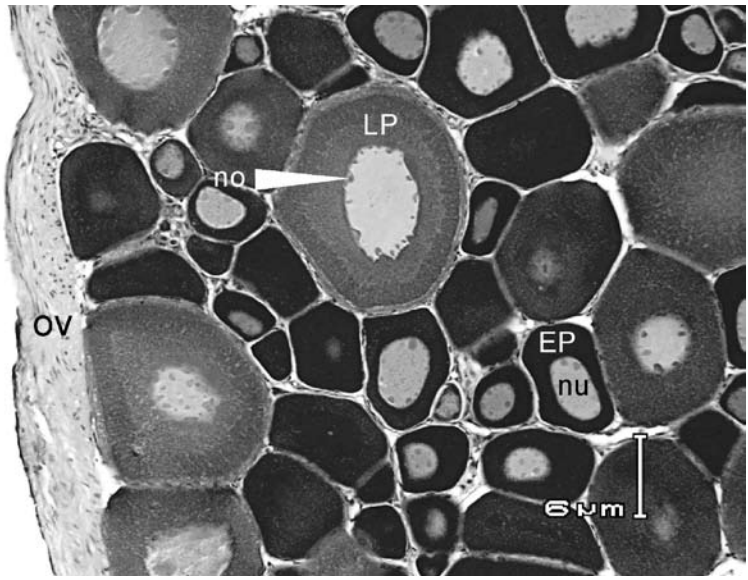

Figure 2. Immature pollock ovary showing the ovarian wall (ov) and containing both early (EP) and late (LP) peri-nucleus oocytes pictured with their nucleus (nu) and nucleolus (no).

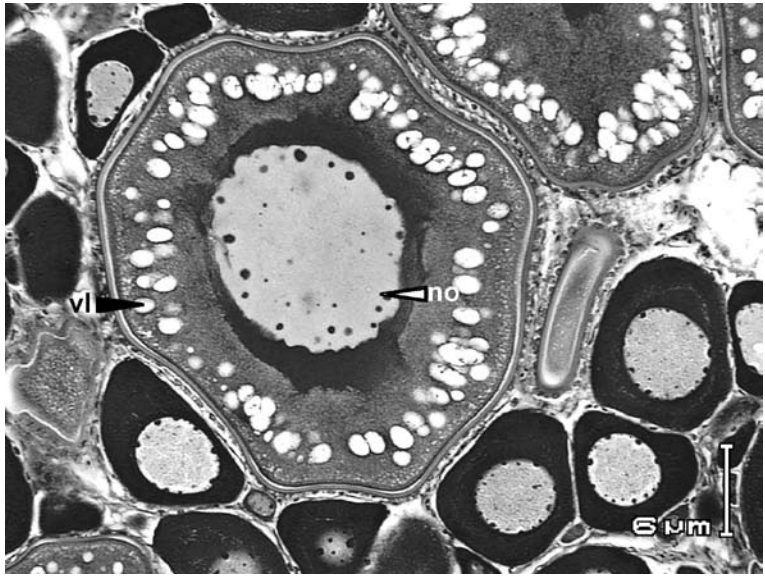

Figure 3. A yolk vesicle oocyte is pictured with its nucleoli (no), and vacuoles (vl).

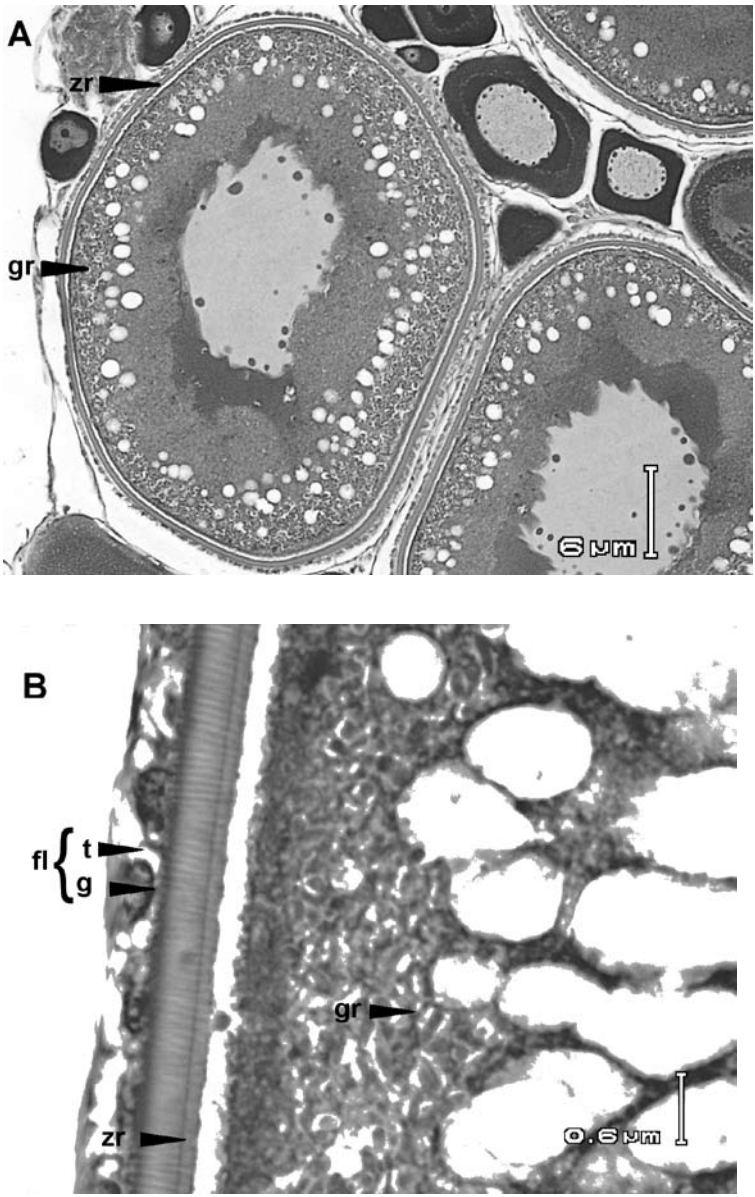

Figure 4. Primary yolk oocyte. The yolk granules (gr) and zona radiata (zr) are pictured for a primary yolk oocyte at (A) 100X and (B) 1,000X. The follicle (fl) and its parts, the granulosa (g) and theca (t), are also shown at 1,000X magnification.

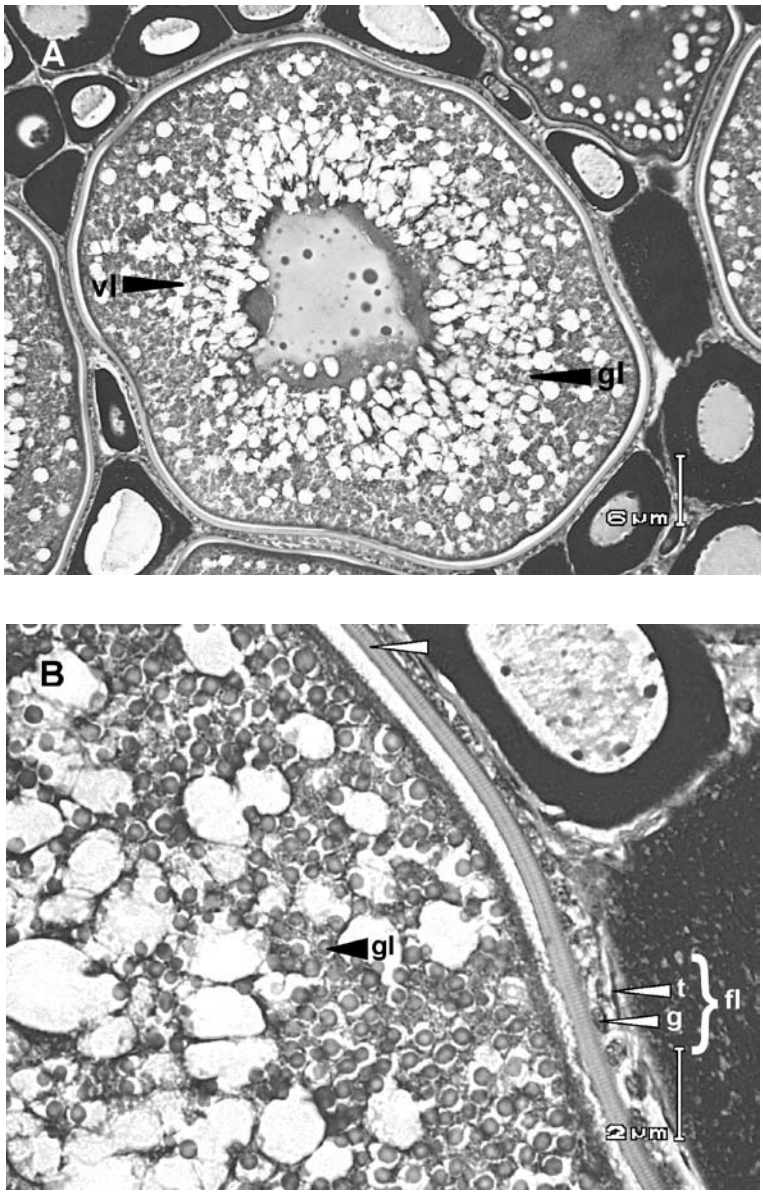

Figure 5. Secondary yolk oocyte. The vacuole (vl), and yolk globules (gl) are shown for a secondary yolk oocyte under (A) 100X and (B) 400X. In addition, the zona radiata (zr) and follicle (fl), including its parts of granulosa (g) and theca cells (t) are labeled for 400X.

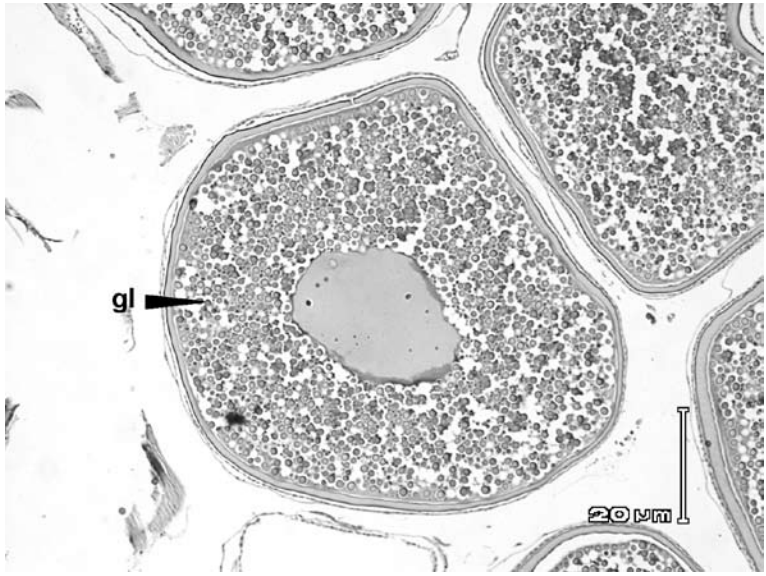

Figure 6. Tertiary yolk oocyte. A tertiary yolk stage oocyte is pictured with its yolk globules (gl).

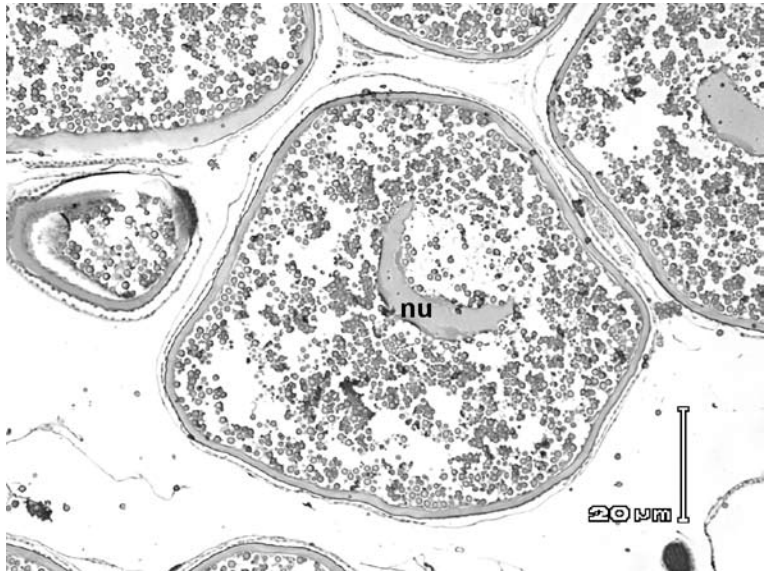

Figure 7. Nuclear migration. An oocyte is pictured at the nuclear migration stage with a crescent-shaped nucleus (nu).

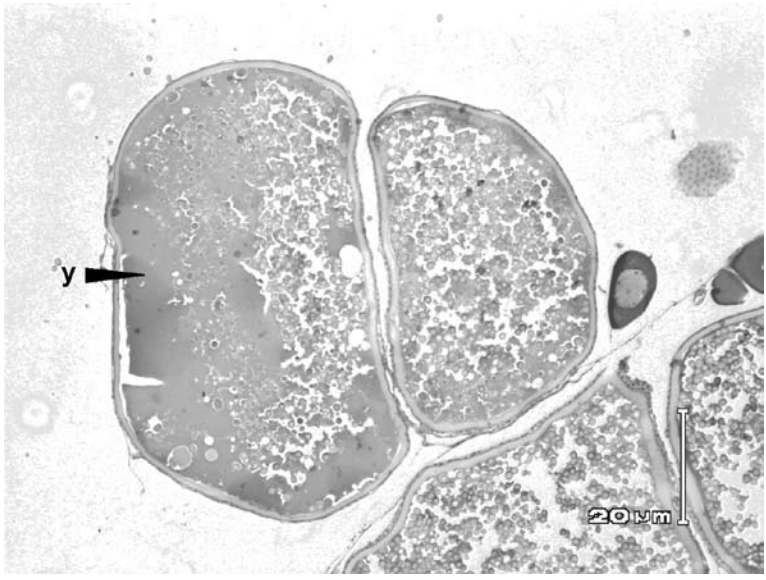

Figure 8. Prematuration. At the prematuration oocyte stage, fused yolk globules (y) are shown.

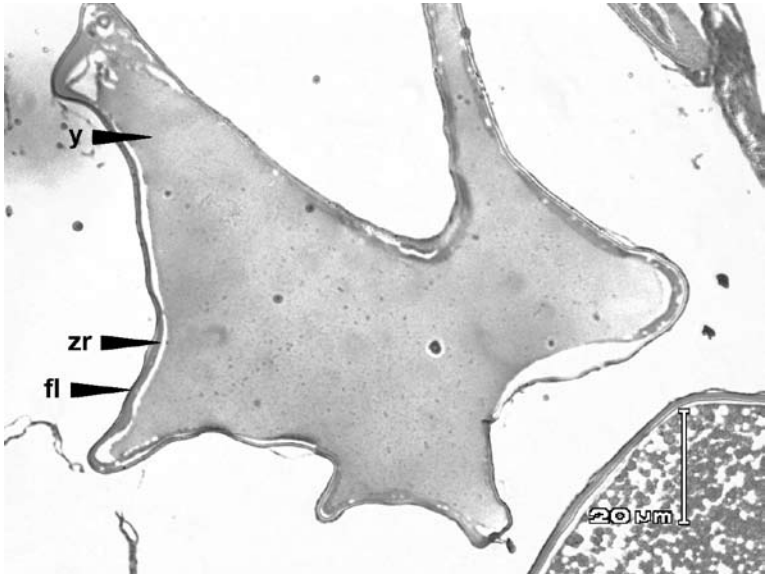

Figure 9. Maturation. The maturation stage is shown with its follicle (fl), fused yolk (y), and zona radiata (zr).

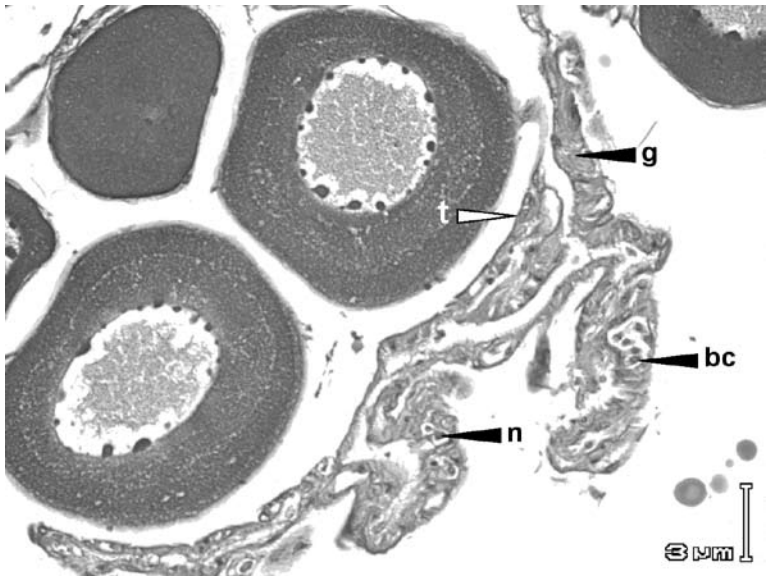

Figure 10. Postovulatory follicle. The nuclei (n), granulosa (g), and theca (t) cells of the degrading follicle are pictured along with blood capillaries (bc).

Results

A GSI was calculated for 158 histological samples of ovaries for which no evidence of atresia was found. When plotted against length, a break in GSI values occurred across all length classes; no GSI values were found between 3.8-6.9 (Fig. 11A, B). All ovaries that contained empty follicles were above this GSI break and their most advanced oocytes were at stages of nuclear migration to ovulation (Fig. 11A). Sixty percent of ovaries above the break did not have empty follicles, but had their most advanced oocytes in the nuclear migration, prematuration, or maturation stages. Below the break, no ovaries had oocytes advanced to the prematuration or maturation stages (Fig. 11A). All ovaries classified by histological analysis as immature, i.e., fish that will not spawn in the approaching season, were below the break and had a GSI value ≤ 1.0 (Fig. 11B). Altogether 74% of ovaries classified as mature were above the break. Ovaries that were below the break and classified as mature were primarily at early yolked stages. The mean ± 1 SD of the GSI for immature fish (0.482 ± 0.190, N = 49) was statistically significantly lower than the mean for mature fish (9.22 ± 4.97, N = 109) using a two-sample *t*-test for unequal variances, $t = -18.2$, $p < 0.001$.

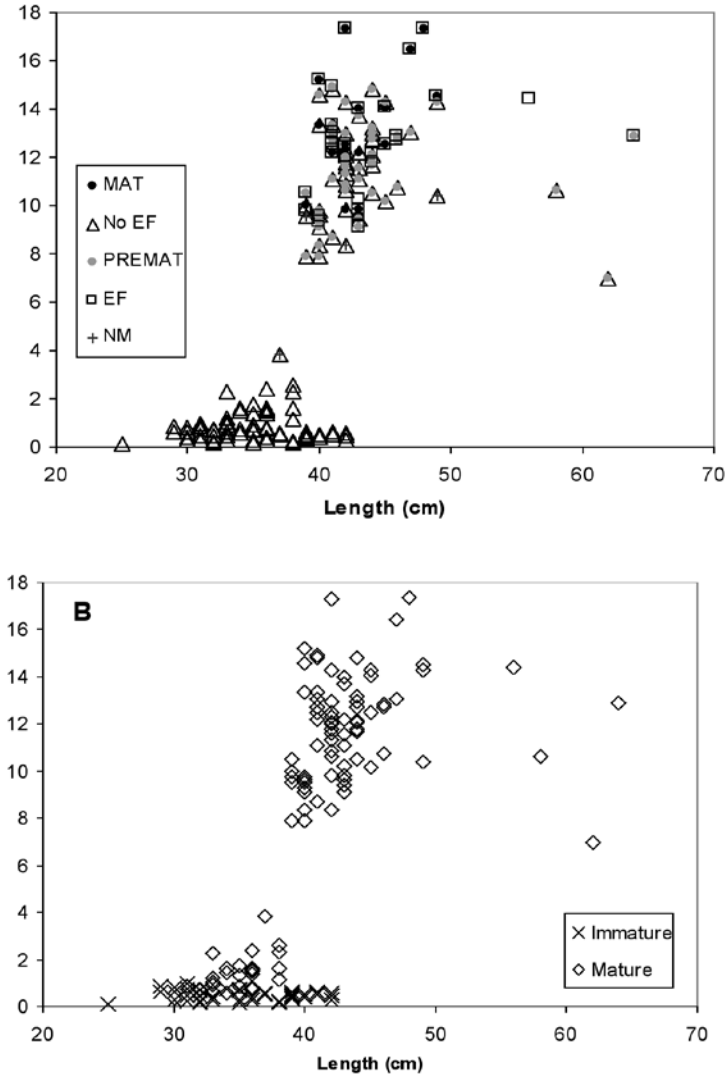

Figure 11. A plot of GSI versus fork length for histological samples collected in 2003 show a break in GSI values at about 6.9 for which ovaries: (A) contain empty follicles (EF) and/or have oocytes at the nuclear migration (NM), prematuration (PREMAT), or maturation (MAT) stages as their most advanced oocytes present; and (B) are classified as immature or mature based on histological examination. No ovaries with atresia were included.

Using macroscopic visual inspection criteria (Table 1), PCC personnel staged 4,865 pollock in 2002 and 5,095 in 2003. The GSI values were calculated and plotted against length for maturity stages 2 through 5. The GSI values were not calculated for immature (maturity stage 1) fish due to inaccuracy of small ovary weights measured by vessels' scales. Developing fish (maturity stage 2) had fork lengths of 24-78 cm and GSI values of 0.16-20 with an average of 4.36. Pre-spawning fish (maturity stage 3) were 28-78 cm fork length with GSI values from 1.32 to 34.43 with an average of 10.89. Spawning fish were 32-80 cm fork length with GSI values of 1.38 to 35.25 with an average of 13.49. Spent fish were 33-73 cm fork length with GSI values from 0.14 to 38.27 with an average at 7.12. Due to the high degree of overlap in GSI values among maturity stages, it was unnecessary to apply ANOVA to show the lack of statistical significance.

Of the 173 pollock ovaries collected for histological analysis, 64 (37%) were macroscopically classified as immature. Of these, the most advanced oocytes of 22% of the ovaries were at early yolked stages and 78% were at less developed stages (peri-nucleus or yolk vesicle). Twenty pollock (12%) were classified as developing; of these, 20% were at early developmental stages of peri-nucleus or yolk vesicle, 70% were early yolked, and 10% were late yolked to nuclear migration stages as their most advanced oocytes present. Eighty-nine pollock (51%) were staged as pre-spawning; 98% of these were at advanced oocyte stages from tertiary yolk to ovulation. No postovulatory follicles were found in ovaries macroscopically staged as immature or developing. Postovulatory follicles were found in 36 ovaries staged as pre-spawning. None of the sampled ovaries were categorized as spawning or spent by macroscopic inspection according to the five-stage scale utilized (Table 1).

The assessed maturity condition of ovaries staged histologically was not always in agreement with the macroscopically determined maturity condition (Table 3). The misclassification rate for fish macroscopically identified as immature was 41%, for developing 95%, and for pre-spawning 44%. After histological examination, maturity condition was concluded to be 59% immature, 19% developing, and 22% pre-spawning for ovaries macroscopically staged as immature; 15% immature, 5% developing, and 80% pre-spawning, for ovaries macroscopically staged as developing; and 56% pre-spawning, 38% spawning, and 6% not staged for ovaries macroscopically staged as pre-spawning. Ovaries were not staged if more than 50% of their oocytes were atretic. If ovaries macroscopically staged as developing are presumed not to spawn in the current year (i.e., immature), then 26% of mature ovaries were misclassified by macroscopic examination as immature and 0% of immature ovaries were misclassified as mature.

Ovary coloration varied with developmental stage. Ovaries with their most advanced oocytes at peri-nucleus or yolk vesicle stages

were transparent or translucent with a highly variable coloration, from colorless to maroon. Those with the primary yolk stage as their most advanced oocytes were translucent and orange or maroon, or opaque and orange. Ovaries with their most advanced oocytes at secondary, tertiary, or nuclear migration stages were opaque and orange and at prematuration, maturation, or ovulation stages were flesh or tan-orange.

Discussion

Our results indicate that GSI may be a good predictor of fish that are about to spawn or have already begun spawning. A break in GSI values separates most mature and immature fish based on histological determinations (Fig. 11). Mature fish above the break had their most advanced oocytes at the nuclear migration to ovulation stages and many had empty follicles (an indication that spawning had begun) (Fig. 11A). At the maturation stage, oocytes take up water during the hydration process and ovary weights may increase two to four fold above those other maturity stages (Hunter and Macewicz 1985). Thus, GSI is useful in identification of hydrated ovaries (Hunter and Macewicz 1985). Ovaries with their most advanced oocytes at nuclear migration or prematuration stages may have occurred above the break, because hydrated oocytes were in the ovary but not observed in the particular ovary sample examined histologically. The hydration stage is very short in duration and may not be commonly observed (Sorokin 1961, Hunter and Goldberg 1980).

Although Sakurai (1989) suggested that pollock that will spawn in the approaching season may be identified by GSI ≥ 2.5, we found some pollock with their most advanced oocyte stages at early yolked, late yolked, or nuclear migration stages with GSI < 2.5; fish with oocytes at these stages are expected to spawn in the approaching season. Moreover, fish classified as developing, pre-spawning, and spawning based on macroscopic examination had average GSI > 2.5, but some individuals had GSI < 2.5. In addition, some immature and mature fish examined histologically had overlapping GSI values below the break (Fig. 11B). For these reasons, GSI cannot be used as the sole predictor of fish that will spawn in the approaching season in the eastern Bering Sea.

The GSI values for spent fish were intermediate between developing and pre-spawning/spawning pollock, consistent with findings of Merati (1993). Sakurai (1989) observed GSI values > 1.0 for post-spawning pollock but this criterion does not conform well to our observations. Although, the average GSI for spent fish in our study was 7.12, some GSI values were below 1.0. In addition, the position of some spent fish above the GSI break (Fig. 11) wrongly suggests post-spawners were either close to spawning or spawning. Consequently, GSI may not be

useful to identify post-spawners, which agrees with findings of Hunter and Macewicz (1985).

Differences or similarities among pollock GSI values between Funka Bay, Japan (Sakurai 1989); Shelikof Strait, Alaska (Merati 1993); and the eastern Bering Sea (this study) must be interpreted cautiously. It is inappropriate to compare GSI values across populations (deVlaming et al. 1982). Relationships between ovary weight and body size may change with development (deVlaming et al. 1982, Hunter and Macewicz 1985, West 1990), therefore samples may be biased when comparing GSI values for fish of different sizes. Larger fish may have larger GSI values than smaller fish at the same maturity stage (Hunter and Macewicz 1985, West 1990), and this effect is augmented during late maturation stages (Hunter and Macewicz 1985). In this regard, Sakurai (1989) calculated GSI values from a smaller range of pollock lengths and a smaller sample size than in our study.

The high rate of misclassification using macroscopic maturity staging is likely to be largely due to the subjectivity of the procedure; determinations are dependent on interpretations of color, size of the ovary and visibility of eggs. Some spent pollock, particularly large fish, may have been misclassified as immature. Instead, some of these large immature fish may have been in a non-reproductive state, as observed in Atlantic cod (Shirokova 1969 as cited by Morrison 1990, Rideout et al. 2000). Fish that skip spawning in a season because they do not commence vitellogenesis or they resorb yolked oocytes may be difficult to distinguish macroscopically from those that are immature (Rideout et al. 2005). Alternatively, because large fish may spawn first as with Atlantic cod (Morrison 1990), they may be more likely to be spent and misclassified as immature during data collection. Pre-spawning pollock will spawn in the current season and developing pollock will spawn in the following season; however, the distinction between the two stages is difficult to make based solely on macroscopic examination. In addition, significant numbers of pre-spawning (maturity stage 3) pollock may be misidentified as developing. Hence, histological analysis is superior, albeit more time consuming, than macroscopic examination, because stages of oocyte development are observed directly.

Additional misclassification errors based on macroscopic maturity staging may be due to errors in the classification scheme itself. For instance, according to criteria of the five-stage scale (Table 1), a spawning pollock has an ovary in which "most" eggs are hydrated. However, presence of hydrated oocytes indicates that spawning either will begin soon or has already begun (Hinckley 1986) because hydration is a brief stage (Sorokin 1961, Hunter and Goldberg 1980). Thus, applying this scale will lead to some fish defined biologically as spawning to be misclassified as pre-spawning. Spawning may be better described macroscopically by the presence of "any" hydrated oocytes. Likewise,

Table 2. Pollock maturity condition based on histological examination. Pollock were classified into a maturity condition based on histological examination of their ovaries (Hinckley 1986; Morrison 1990; Y. Sakurai, Hokkaido University, pers. comm.).

Maturity condition	Histological examination
I. Immature	Late peri-nucleus (Fig. 2) most advanced oocyte stage present. No postovulatory follicles.
II. Developing	Yolk vesicle stage (Fig. 3) most advanced oocyte stage present. Early and late peri-nucleus stages may also be present. No yolk formation. No postovulatory follicles.
III. Pre-spawning	Peri-nucleus, primary (Fig. 4), secondary (Fig. 5), and tertiary yolked (Fig. 6), nuclear migration (Fig. 7), and prematuration stages (Fig. 8) may be present, but maturation most advanced oocyte stage present (Fig. 9). No postovulatory follicles.
IV. Spawning	Ovulation has occurred. Peri-nucleus, yolked, nuclear migration, prematuration, and maturation stages may be present. Postovulatory follicles present (Fig. 10).
V. Spent	No oocytes beyond peri-nucleus stage. Atresia of unspawned maturation stage oocytes may be present. Postovulatory follicles present.

coloration of pollock ovaries may be indicative of not only developmental stage, but also diet (J.F. Morado, NMFS, Seattle, pers. comm.) and perhaps other factors. Yolk begins to form as granules in the primary yolk stage (Hinckley 1986). Pollock ovaries with their most advanced oocytes at the primary yolk to nuclear migration stages, exhibited an orange coloration, indicating enough yolk had formed to affect the color of the ovary. We observed high color variation in ovaries that had their most advanced oocyte stages at the peri-nucleus, yolk vesicle, and primary yolk stages. Pollock ovaries at the immature, developing, and pre-spawning I stages may be more subject to color variability due to diet, because not enough yolk has formed to mask the influence of diet on coloration.

Classification of ovary development based on histology (Table 2) is not without other caveats. For instance, criteria to classify fish into the pre-spawning stage based on histology (Table 2) was based on the assumption that development from the primary yolk stage to spawning occurs in four months as in Funka Bay, Japan (Y. Sakurai, Hokkaido University, pers. comm.). However, development time in the eastern Bering Sea is unknown and may differ due to differences in temperatures or genotypes. Spawning in the eastern Bering Sea has been recorded from March to June (Hinckley 1986). Based on the assumption about ovarian development and given the spawning schedule, females

Table 3. Ovary classification matrix based on macroscopic and histological examination. Ovaries collected in 2003 were placed into a maturity condition based only on macroscopic inspection and then reclassified after histological examination. The rate of misclassification for macroscopic staging by developmental stage is illustrated. An ovary was not staged if more than 50% of its oocytes were atretic.

Histological determinations	Macroscopic determinations		
	Immature	Developing	Pre-spawning
Immature	38	3	0
Developing	12	1	0
Pre-spawning	14	15	13
Spawning	0	1	71
Not staged	0	0	5

collected in the winter (late January to early April) with their most advanced oocytes at stages of primary yolk or beyond should spawn the same calendar year in the eastern Bering Sea or elsewhere in the Bering Sea. Thus, pollock categorized as immature or developing are not expected to spawn until the following season. Therefore, 22% of the pollock macroscopically classified as immature using criteria in Table 1 and the 80% classified as developing would spawn in the year of our data collection, but after the "A" fishing season. In addition, the time of development from peri-nucleus or yolk vesicle stages to spawning and the duration of each oocyte stage is unknown.

Sample size may have affected our results, as well. The sample size of ovaries used for histological examination was relatively small and was limited both temporally and spatially collected from only a few haul locations near the Pribilof Islands. The schedule of ovary development may vary by region due to temperature differences, by age class due to differences in thermal histories (T.W. Buckley, NOAA, Seattle, pers. comm.), and by populations owing to genetic variation in life history characteristics (Olsen et al. 2004). Interannual differences in development rates of Atlantic cod gonads are correlated with temperature and oxygen content of seawater (Uzars et al. 2001).

A new descriptive guide, including photographs and descriptions of ovaries at each stage was produced for more accurate macroscopic staging of pollock ovaries (Appendix). The descriptions of each maturity condition were based on macroscopic observation, histological analysis,

and previously developed guides (Hinckley 1986; Bowden et al. 1990; and N.J. Williamson, NMFS, Seattle, pers. comm.).

Difficulties in applying macroscopic determinations of maturity stages of pollock suggest the need for additional research. Histological examination should be performed for ovaries collected from locations across the eastern Bering Sea over several years to determine if the relationship between macroscopic and histological staging varies temporally or spatially. Laboratory studies should be performed to determine the time from development of various oocyte stages, such as from primary yolk to spawning, to determine potential differences among regions and over different thermal regimes experienced by pollock. Such a study is necessary to verify which fish will spawn in the approaching season and which will spawn in the subsequent season and to validate descriptive guides, such as ours. Fieldwork, such as tagging and sampling of fish in late spring or early summer, might determine if some pollock caught on the eastern Bering Sea shelf during the "A" fishing season spawn after the suggested spawning season and/or if they spawn elsewhere in the Bering Sea. More accurate classifications of mature or immature pollock are necessary for accurate estimation of size of maturity and spawning stock biomass for fishery management. We will address this last issue in a forthcoming paper.

Acknowledgments

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Appendix: Descriptive guide to macroscopic staging of pollock ovaries

The following guide is intended to aid in the classification of pollock ovaries into accurate maturity stages based on visual macroscopic inspection. Photographs of ovaries and descriptions of ovary coloration and size are provided for each maturity stage. Additional information is provided on the topics of ovary coloration, ovary size, and atresia. Macroscopic staging criteria were developed based on personal observation and criteria from previously developed guides (Hinckley 1986; Bowden et al. 1990; N.J. Williamson, NMFS, Seattle, pers. comm.).

Assumptions

This guide is based on the assumption that pollock with ovaries classified in the "A" fishing season as pre-spawning will spawn within the current year and those with developing ovaries will spawn the following year. Ovaries were placed into the pre-spawning macroscopic maturity stage if they had oocytes of primary yolk or more advanced oocyte stages, because time of development from primary yolk stage to spawning occurs in four months in Funka Bay, Japan (Y. Sakurai, Hokkaido University, pers. comm.). However, the time of development from each histological stage to spawning is unknown in the eastern Bering Sea. Laboratory studies are needed to verify consistency with the time frame of development in Funka Bay or to determine an alternative. This guide would change if an alternative time frame were found for development.

The duration of each oocyte stage is unknown; therefore, an assumption of a short time interval is made. Otherwise, an ovary with oocytes at the beginning of the primary yolk stage may be classified differently from an ovary with oocytes at the end of this stage. Laboratory work is needed to verify the duration of each oocyte stage.

The photographed ovaries were not matched one to one with a histology classification, so some uncertainty exists in correct placement of each ovary into a macroscopic visual maturity category.

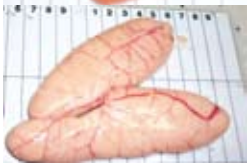
1. Immature: Ovaries transparent or translucent, colorless, gray, yellow, orange, orange-yellow, pink, maroon, reddish-orange, tan, or tan-orange. No eggs visible. Ovary small and tucked inside body cavity.

2. Developing: Picture is unavailable. Ovary translucent, orange, pink, maroon, reddish-orange, or tan-orange. No eggs visible. Ovary small. Likely to spawn the following year.

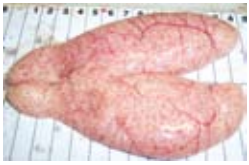
3. Pre-spawning:

I. (presumptive) Ovary translucent, orange, pink-orange, maroon, or tan-orange. No eggs visible to the eye. Ovary less than ½ length of the body cavity. Well-developed red blood vessels. Likely to spawn later this year.

II. Ovary opaque, orange, bright orange, or dark orange. Visible eggs. Ovary occupies about ½ of ventral cavity.



III. Ovary opaque, tan-orange or flesh colored. Visible eggs. Ovary occupies about ⅔ of the body cavity.



4. Spawning: Some to most eggs are translucent from hydration. Ovary occupies whole body cavity. Other eggs are opaque and tan-orange or flesh colored. Ovary must be cut through center, where spawning begins, to check for hydrated eggs. Ova may run with pressure. Thin, stretched ovarian wall.

5. Spent: Ovaries empty and flaccid. Thick ovarian wall. (Photograph by Sarah Hinckley, NMFS.)

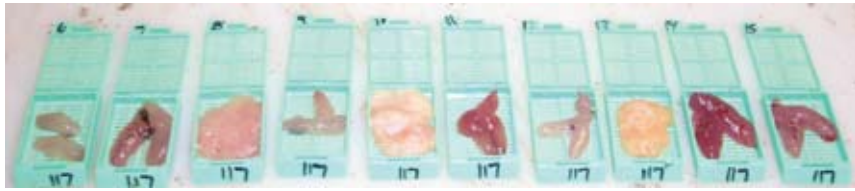


Figure A1. Color variation observed in visual stage 1 and stage 3 ovaries is pictured. Microcassette dimensions are approximately 2.5 cm x 3.8 cm.

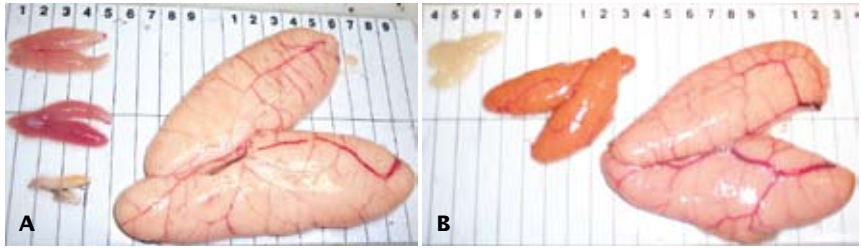


Figure A2. Ovary sizes: (A) Pre-spawning I ovaries are pictured on the top left and center left, immature on the bottom left, and pre-spawning III on the right. (B) Immature ovaries are shown on the left, pre-spawning II in the center, and pre-spawning III on the right.

Coloration

The described coloration of the ovaries at each developmental stage in this guide was observed, but other colors may occur. Color may be affected not only by the amount of yolk within the oocytes, but also by diet. Immature, developing, and early pre-spawning ovaries may be subject to more variability due to diet, because not enough yolk has formed to mask the influence of diet on coloration (Fig. A1).

Size

Ovaries become larger with yolk formation and development and very large with absorption of water with hydration (Fig. A2. A, B).

Atresia

Ovaries with atretic oocytes may appear at advanced developmental stages under macroscopic inspection; however, they may only contain early developmental stages or atretic oocytes.